=> d his

(FILE 'HOME' ENTERED AT 17:10:58 ON 14 NOV 2003)

FILE 'MEDLINE' ENTERED AT 17:11:11 ON 14 NOV 2003
E UNDERHILL?/AU
E UNDERHILL T?/AU
L1 8 S E1
L2 133 S PGL3?
L3 0 S L2 AND SOX9?
L4 340 S SOX9
L5 3 S L4 AND L1
L6 88 S L4 AND COLLAGEN?
L7 27 S L6 AND ENHANCER?
L8 21 S L7 AND COL2A?
L9 21 SORT L8 PY
L10 21 S L9
L11 13 S L9 AND PY<=2000

FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, MEDICONF' ENTERED AT 17:23:01 ON 14 NOV 2003
L12 54 S SOX9 (L) COLLAGEN? (L) COL2? (L) ENHANCER?
L13 19 DUP REM L12 (35 DUPLICATES REMOVED)
L14 19 SORT L13 PY
L15 5 S L14 AND 48(W)BP
L16 5 SORT L15 PY

=> d an ti so au ab pi l16 1-5

L16 ANSWER 1 OF 5 MEDLINE on STN
AN 1998279015 MEDLINE
TI Chondrocyte-specific enhancer elements in the Col11a2 gene resemble the Col2a1 tissue-specific enhancer.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Jun 12) 273 (24) 14998-5006. Journal code: 2985121R. ISSN: 0021-9258.
AU Bridgewater L C; Lefebvre V; de Crombrugghe B
AB Type XI **collagen** and type II **collagen** are coexpressed in all cartilage, and both are essential for normal cartilage differentiation and skeletal morphogenesis. This laboratory has recently identified a 48-base pair (bp) **enhancer** element in the type II **collagen** gene **Col2a1** that contains several HMG-type protein-binding sites and that can direct chondrocyte-specific expression in transient transfection and in transgenic mice. The present study has identified two short chondrocyte-specific **enhancer** elements within a region in the 5' portion of the type XI **collagen** gene Col11a2 that has previously been shown to influence chondrocyte-specific expression in transgenic mice. These Col11a2 **enhancer** elements, like the **Col2a1 enhancer**, contain several sites with homology to the high mobility group (HMG) protein-binding consensus sequence. In electrophoretic mobility shift assays, the Col11a2 elements formed a DNA-protein complex that was dependent on the presence of the HMG-like sites. It had the same mobility as the complex formed with the **Col2a1 48-bp enhancer** and appeared to contain the same or similar proteins, including **SOX9**. The Col11a2 elements directed gene expression in transient transfections of chondrocytes but not fibroblasts, and their activity was abolished by mutation of the HMG-like sites. Ectopically expressed **SOX9** activated these **enhancers** in non-chondrocytic cells, as it also activates the **Col2a1 enhancer**. Finally, the Col11a2 **enhancer** elements both directed transgene expression to cartilage in developing mouse embryos. Overall, our results indicate that the two Col11a2 chondrocyte-specific **enhancer** elements share many similarities with the **Col2a1 48-bp enhancer**. These similarities suggest the existence of a genetic program designed to coordinately regulate the expression of these and perhaps other genes involved in the chondrocyte differentiation pathway.

L16 ANSWER 2 OF 5 MEDLINE on STN
AN 1998279014 MEDLINE
TI Three high mobility group-like sequences within a 48-base pair enhancer of

the Col2a1 gene are required for cartilage-specific expression in vivo.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Jun 12) 273 (24) 14989-97.
Journal code: 2985121R. ISSN: 0021-9258.
AU Zhou G; Lefebvre V; Zhang Z; Eberspaecher H; de Crombrugghe B
AB To understand the molecular mechanisms by which mesenchymal cells differentiate into chondrocytes, we have used the gene for an early and abundant marker of chondrocytes, the mouse pro-alpha1(II) **collagen** gene (**Col2a1**), to delineate a minimal sequence needed for chondrocyte-specific expression and to identify the DNA-binding proteins that mediate its activity. We show here that a 48-base pair (bp) **Col2a1** intron 1 sequence specifically targets the activity of a heterologous promoter to chondrocytes in transgenic mice. Mutagenesis studies of this **48-bp** element identified three separate sites (sites 1-3) that were essential for its chondrocyte-specific **enhancer** activity in both transgenic mice and transient transfections. Mutations in sites 1 and 2 also severely inhibited the chondrocyte-specific **enhancer** activity of a 468-bp **Col2a1** intron 1 sequence in vivo. **SOX9**, an SRY-related high mobility group (HMG) domain transcription factor, was previously shown to bind site 3, to bend the **48-bp** DNA at this site, and to strongly activate this **48-bp** **enhancer** as well as larger **Col2a1 enhancer** elements. All three sites correspond to imperfect binding sites for HMG domain proteins and appear to be involved in the formation of a large chondrocyte-specific complex between the **48-bp** element, **Sox9**, and other protein(s). Indeed, mutations in each of the three HMG-like sites of the **48-bp** element, which abolished chondrocyte-specific expression of reporter genes in transgenic mice and in transiently transfected cells, inhibited formation of this complex. Overall our results suggest a model whereby both **Sox9** and these other proteins bind to several HMG-like sites in the **Col2a1** gene to cooperatively control its expression in cartilage.

L16 ANSWER 3 OF 5 MEDLINE on STN
AN 2001019679 MEDLINE
TI Transcriptional mechanisms of chondrocyte differentiation.
SO MATRIX BIOLOGY, (2000 Sep) 19 (5) 389-94. Ref: 25
Journal code: 9432592. ISSN: 0945-053X.
AU de Crombrugghe B; Lefebvre V; Behringer R R; Bi W; Murakami S; Huang W
AB With the goal of identifying master transcription factors that control the genetic program of differentiation of mesenchymal cells into chondrocytes, we first delineated a **48-bp** chondrocyte-specific **enhancer** element in the gene for proalpha1(II) **collagen** (**Col2a1**), an early and abundant marker of chondrocytes. Our experiments have demonstrated that the HMG-box-containing transcription factor, **Sox9** which binds and activates this **enhancer** element, is required for chondrocyte differentiation and for expression of a series of chondrocyte-specific marker genes including **Col2a1**, Col9a2, Col11a2 and Aggrecan. In the absence of **Sox9** the block in differentiation occurs at the stage of mesenchymal condensation, suggesting the hypothesis that **Sox9** might also control expression of cell surface proteins needed for mesenchymal condensation. Since **Sox9** also contains a potent transcription activation domain, it is a typical transcription factor. Two other members of the Sox family, L-Sox5 and Sox6, also bind to the **48-bp** **Col2a1 enhancer** and together with **Sox9** activate this **enhancer** as well as the endogenous **Col2a1** and aggrecan genes. L-Sox5 and Sox6 have a high degree of sequence identity to each other and are likely to have redundant functions. Except for the HMG-box, L-Sox5 and Sox6 have no similarity to **Sox9** and, hence, are likely to have a complementary function to that of **Sox9** . Our experiments suggest the hypothesis that, like **Sox9**, Sox5 and Sox6 might also be needed for chondrocyte differentiation. Other experiments, have provided evidence that the **Sox9** polypeptide and the **Sox9** gene are targets of signaling molecules that are known to control discrete steps of chondrogenesis in the growth plate of endochondral bones. Protein kinase A (PKA) phosphorylation of **Sox9** increases its DNA binding and transcriptional activity. Since PKA-phosphorylated-**Sox9** is found in the prehypertrophic

zone of the growth plate, the same location where the gene for the receptor of the parathyroid hormone-related peptide (PTHrP) is expressed and since PTHrP signaling is mediated by cyclic AMP, we have hypothesized that **Sox9** is a target for PTHrP signaling. Other experiments have also shown that fibroblast growth factors (FGFs) increase the expression of **Sox9** in chondrocytes in culture and that this activation is mediated by the mitogen-activated protein kinase pathway. These results favor the hypothesis that in achondroplasia, a disease caused by activating mutations in FGF receptor 3, there might also be an abnormally high **Sox9** expression.

L16 ANSWER 4 OF 5 MEDLINE on STN
AN 2001572482 MEDLINE
TI L-Sox5, Sox6 and Sox9 control essential steps of the chondrocyte differentiation pathway.
SO OSTEOARTHRITIS AND CARTILAGE, (2001) 9 Suppl A S69-75.
Journal code: 9305697. ISSN: 1063-4584.
AU Lefebvre V; Behringer R R; de Crombrugghe B
AB OBJECTIVE: This work was carried out to identify transcription factors controlling the differentiation of mesenchymal cells into chondrocytes. DESIGN: We delineated a cartilage-specific **enhancer** in the **collagen** type 2 gene (**Col2a1**) and identified transcription factors responsible for the activity of this **enhancer** in chondrocytes. We then analyzed the ability of these transcription factors to activate specific genes of the chondrocyte differentiation program and control cartilage formation in vivo. RESULTS: A **48-bp** sequence in the first intron of **Col2a1** drove gene expression specifically in cartilage in transgenic mouse embryos. The transcription factors L-Sox5, Sox6, and **Sox9** bound and cooperatively activated this **enhancer** in vitro. They belong to the Sry-related family of HMG box DNA-binding proteins, which includes many members implicated in cell fate determination in various lineages. L-Sox5, Sox6, and **Sox9** were coexpressed in all precartilaginous condensations in mouse embryos and continued to be expressed in chondrocytes until the cells underwent final hypertrophy. Whereas L-Sox5 and Sox6 are highly homologous proteins, they are totally different from **Sox9** outside the HMG box domain. The three proteins cooperatively activated the **Col2a1**- and aggrecan genes in cultured cells. Heterozygous mutations in **SOX9** in humans lead to campomelic dysplasia, a severe and generalized skeletal malformation syndrome. Embryonic cells with a homozygous **Sox9** mutation were unable to form cartilage in vivo and activate essential chondrocyte marker genes. Preliminary data indicated that the mutation of Sox5 and Sox6 in the mouse led to severe skeletal malformations. CONCLUSIONS: L-Sox5, Sox6, and **Sox9** play essential roles in chondrocyte differentiation and, thereby, in cartilage formation. Their discovery will help to understand further the molecular mechanisms controlling chondrogenesis in vivo, uncover genetic mechanisms underlying cartilage diseases, and develop novel strategies for cartilage repair.

L16 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2002:969453 CAPLUS
DN 138:266673
TI A New Long Form of c-Maf Cooperates with Sox9 to Activate the Type II Collagen Gene
SO Journal of Biological Chemistry (2002), 277(52), 50668-50675
CODEN: JBCHA3; ISSN: 0021-9258
AU Huang, Wendong; Lu, Ni; Eberspaecher, Heidi; de Crombrugghe, Benoit
AB A new long form of the c-Maf transcription factor (Lc-Maf) was identified and shown to interact specifically with SOX9 in a yeast two-hybrid cDNA library screening. Lc-Maf encodes an extra 10 amino acids at the carboxyl terminus of c-Maf and contains a different 3'-untranslated region compared with c-Maf. The interaction between SOX9 and Lc-Maf was further confirmed by co-immunopptn. and glutathione S-transferase pull-down assays, which mapped the interacting domain of SOX9 to the high mobility group box DNA binding domain and that of Lc-Maf to the basic leucine zipper motif. In situ hybridizations showed that Lc-Maf RNA was coexpressed with Sox9 and Col2a1 RNA in areas of precartilaginous mesenchymal condensations during mouse embryo development. A DNA binding site of Lc-Maf was identified at the 5'-end of a **48-bp** Col2a1 enhancer element near the

high mobility group binding site of SOX9. Lc-Maf and SOX9 synergistically activated a luciferase reporter plasmid contg. a Col2a1 enhancer and increased the transcription of the endogenous Col2a1 gene. In summary, Lc-Maf is the first transcription factor shown to interact with Sox9, to be coexpressed with Sox9 during an early step of chondrogenesis and to cooperate with Sox9 in activating a downstream target gene of Sox9.

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(FILE 'HOME' ENTERED AT 17:10:58 ON 14 NOV 2003)

FILE 'MEDLINE' ENTERED AT 17:11:11 ON 14 NOV 2003
E UNDERHILL?/AU
E UNDERHILL T?/AU
L1 8 S E1
L2 133 S PGL3?
L3 0 S L2 AND SOX9?
L4 340 S SOX9
L5 3 S L4 AND L1
L6 88 S L4 AND COLLAGEN?
L7 27 S L6 AND ENHANCER?
L8 21 S L7 AND COL2A?
L9 21 SORT L8 PY
L10 21 S L9
L11 13 S L9 AND PY<=2000

=> d an ti so au ab l11 1-13

L11 ANSWER 1 OF 13 MEDLINE on STN
AN 2001019679 MEDLINE
TI Transcriptional mechanisms of chondrocyte differentiation.
SO MATRIX BIOLOGY, **(2000 Sep)** 19 (5) 389-94. Ref: 25
Journal code: 9432592. ISSN: 0945-053X.
AU de Crombrugghe B; Lefebvre V; Behringer R R; Bi W; Murakami S; Huang W
AB With the goal of identifying master transcription factors that control the genetic program of differentiation of mesenchymal cells into chondrocytes, we first delineated a 48-bp chondrocyte-specific **enhancer** element in the gene for proalpha1(II) **collagen** (**Col2a1**), an early and abundant marker of chondrocytes. Our experiments have demonstrated that the HMG-box-containing transcription factor, **Sox9** which binds and activates this **enhancer** element, is required for chondrocyte differentiation and for expression of a series of chondrocyte-specific marker genes including **Col2a1**, Col9a2, Col11a2 and Aggrecan. In the absence of **Sox9** the block in differentiation occurs at the stage of mesenchymal condensation, suggesting the hypothesis that **Sox9** might also control expression of cell surface proteins needed for mesenchymal condensation. Since **Sox9** also contains a potent transcription activation domain, it is a typical transcription factor. Two other members of the Sox family, L-Sox5 and Sox6, also bind to the 48-bp **Col2a1** **enhancer** and together with **Sox9** activate this **enhancer** as well as the endogenous **Col2a1** and aggrecan genes. L-Sox5 and Sox6 have a high degree of sequence identity to each other and are likely to have redundant functions. Except for the HMG-box, L-Sox5 and Sox6 have no similarity to **Sox9** and, hence, are likely to have a complementary function to that of **Sox9**. Our experiments suggest the hypothesis that, like **Sox9**, Sox5 and Sox6 might also be needed for chondrocyte differentiation. Other experiments, have provided evidence that the **Sox9** polypeptide and the **Sox9** gene are targets of signaling molecules that are known to control discrete steps of chondrogenesis in the growth plate of endochondral bones. Protein kinase A (PKA) phosphorylation of **Sox9** increases its DNA binding and transcriptional activity. Since PKA-phosphorylated-**Sox9** is found in the prehypertrophic zone of the growth plate, the same location where the gene for the receptor of the parathyroid hormone-related peptide (PTHrP) is expressed and since PTHrP signaling is mediated by cyclic AMP, we have hypothesized that **Sox9** is a target for PTHrP signaling. Other experiments have also shown that fibroblast growth factors (FGFs) increase the expression of **Sox9** in chondrocytes in culture and that this activation is mediated by the mitogen-activated protein kinase pathway. These results favor the hypothesis that in achondroplasia, a disease caused by activating mutations in FGF receptor 3, there might also be an abnormally high **Sox9** expression.

L11 ANSWER 2 OF 13 MEDLINE on STN
AN 2000285447 MEDLINE

TI A zinc finger transcription factor, alphaA-crystallin binding protein 1, is a negative regulator of the chondrocyte-specific **enhancer** of the alpha1(II) **collagen** gene.

SO MOLECULAR AND CELLULAR BIOLOGY, **(2000 Jun)** 20 (12) 4428-35. Journal code: 8109087. ISSN: 0270-7306.

AU Tanaka K; Matsumoto Y; Nakatani F; Iwamoto Y; Yamada Y

AB Transcription of the type II **collagen** gene (**Col2a1**) is regulated by multiple cis-acting sites. The **enhancer** element, which is located in the first intron, is necessary for high-level and cartilage-specific expression of **Col2a1**. A mouse limb bud cDNA expression library was screened by the Saccharomyces cerevisiae one-hybrid screening method to identify protein factors bound to the **enhancer**. A zinc finger protein, alphaA-crystallin binding protein 1 (CRYBP1), which had been reported to bind to the mouse alphaA-crystallin gene promoter, was isolated. We herein demonstrate that CRYBP1 is involved in the negative regulation of **Col2a1 enhancer** activity. CRYBP1 mRNA expression was downregulated during chondrocyte differentiation in vitro. In situ hybridization analysis of developing mouse cartilage showed that CRYBP1 mRNA was also downregulated during mesenchymal condensation and that CRYBP1 mRNA was highly expressed by hypertrophic chondrocytes, but at very low levels by resting and proliferating chondrocytes. Expression of recombinant CRYBP1 in a transfected rat chondrosarcoma cell line inhibited **Col2a1 enhancer** activity. Electrophoretic mobility shift assays showed that CRYBP1 bound a specific sequence within the **Col2a1 enhancer** and inhibited the binding of **Sox9**, an activator for **Col2a1**, to the **enhancer**. Cotransfection of CRYBP1 with **Sox9** into BALB/c 3T3 cells inhibited activation of the **Col2a1 enhancer** by **Sox9**. These results suggest a novel mechanism that negatively regulates cartilage-specific expression of **Col2a1**.

L11 ANSWER 3 OF 13 MEDLINE on STN

AN 2000266302 MEDLINE

TI Phosphorylation of **SOX9** by cyclic AMP-dependent protein kinase A enhances **SOX9**'s ability to transactivate a **Col2a1** chondrocyte-specific **enhancer**.

SO MOLECULAR AND CELLULAR BIOLOGY, **(2000 Jun)** 20 (11) 4149-58. Journal code: 8109087. ISSN: 0270-7306.

AU Huang W; Zhou X; Lefebvre V; de Crombrugghe B

AB **Sox9** is a high-mobility-group domain-containing transcription factor required for chondrocyte differentiation and cartilage formation. We used a yeast two-hybrid method based on Son of Sevenless (SOS) recruitment to screen a chondrocyte cDNA library and found that the catalytic subunit of cyclic AMP (cAMP)-dependent protein kinase A (PKA-Calpha) interacted specifically with **SOX9**. Next we found that two consensus PKA phosphorylation sites within **SOX9** could be phosphorylated by PKA in vitro and that **SOX9** could be phosphorylated by PKA-Calpha in vivo. In COS-7 cells cotransfected with PKA-Calpha and **SOX9** expression plasmids, PKA enhanced the phosphorylation of wild-type **SOX9** but did not affect phosphorylation of a **SOX9** protein in which the two PKA phosphorylation sites (S(64) and S(211)) were mutated. Using a phosphospecific antibody that specifically recognized **SOX9** phosphorylated at serine 211, one of the two PKA phosphorylation sites, we demonstrated that addition of cAMP to chondrocytes strongly increased the phosphorylation of endogenous **Sox9**. In addition, immunohistochemistry of mouse embryo hind legs showed that **Sox9** phosphorylated at serine 211 was principally localized in the prehypertrophic zone of the growth plate, corresponding to the major site of expression of the parathyroid hormone-related peptide (PTHrP) receptor. Since cAMP has previously been shown to effectively increase the mRNA levels of **Col2a1** and other specific markers of chondrocyte differentiation in culture, we then asked whether PKA phosphorylation could modulate the activity of **SOX9**. Addition of 8-bromo-cAMP to chondrocytes in culture increased the activity of a transiently transfected **SOX9**-dependent 48-bp **Col2a1** chondrocyte-specific **enhancer**; similarly, cotransfection of PKA-Calpha increased the activity of this **enhancer**. Mutations of the two PKA phosphorylation consensus sites of **SOX9** markedly

decreased the PKA-Calpha activation of this **enhancer** by **SOX9**. PKA phosphorylation and the mutations in the consensus PKA phosphorylation sites of **SOX9** did not alter its nuclear localization. In vitro phosphorylation of **SOX9** by PKA resulted in more efficient DNA binding. We conclude that **SOX9** is a target of cAMP signaling and that phosphorylation of **SOX9** by PKA enhances its transcriptional and DNA-binding activity. Because PTHrP signaling is mediated by cAMP, our results support the hypothesis that **Sox9** is a target of PTHrP signaling in the growth plate and that the increased activity of **Sox9** might mediate the effect of PTHrP in maintaining the cells as nonhypertrophic chondrocytes.

L11 ANSWER 4 OF 13 MEDLINE on STN
AN 2000160725 MEDLINE
TI **Sox9** expression during chondrogenesis in micromass cultures of embryonic limb mesenchyme.
SO EXPERIMENTAL CELL RESEARCH, **(2000 Mar 15)** 255 (2) 327-32.
Journal code: 0373226. ISSN: 0014-4827.
AU Kulyk W M; Franklin J L; Hoffman L M
AB **Sox9** plays a crucial role in chondrogenesis. It encodes an HMG-domain transcription factor that activates an **enhancer** in the gene for type II **collagen** (**Col2a1**), a principal cartilage matrix protein. We have characterized the temporal pattern of **Sox9** RNA expression in micromass culture, a widely used in vitro model for the analysis of embryonic cartilage differentiation. Cultures were prepared from distal subridge mesenchyme of the stage 24/25 chick embryo wing bud, which undergoes uniform chondrogenic differentiation in vitro. The early "prechondrogenic" phase of culture was characterized by the activation of **Sox9** RNA expression, which preceded detectable upregulation of **Col2a1** transcription. **Sox9** RNA levels peaked between 20 and 65 h of culture, a phase of progressive **Col2a1** transcript accumulation, then declined in the mature cartilage of 120-h cultures. Staurosporine treatment enhanced chondrogenesis in micromass culture by inducing a rapid quantitative increase in **Sox9** transcript levels. However, PMA, a phorbol ester that inhibits **Col2a1** expression and chondrocyte differentiation, had an unexpectedly modest effect on **Sox9** RNA accumulation.

L11 ANSWER 5 OF 13 MEDLINE on STN
AN 2000119334 MEDLINE
TI Potent inhibition of the master chondrogenic factor **Sox9** gene by interleukin-1 and tumor necrosis factor-alpha.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, **(2000 Feb 4)** 275 (5) 3687-92.
Journal code: 2985121R. ISSN: 0021-9258.
AU Murakami S; Lefebvre V; de Crombrugghe B
AB The inflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-alpha) strongly inhibit the expression of genes for cartilage extracellular matrix proteins. We have recently obtained genetic evidence indicating that the high mobility group domain containing transcription factor **Sox9** is required for cartilage formation and for expression of chondrocyte-specific genes including the gene for type II **collagen** (**Col2a1**). We show here that IL-1 and TNF-alpha cause a marked and rapid decrease in the levels of **Sox9** mRNA and/or protein in chondrocytes. A role for the transcription factor NFkappaB in **Sox9** down-regulation was suggested by the ability of pyrrolidine dithiocarbamate, an inhibitor of the NFkappaB pathway, to block the effects of IL-1 and TNF-alpha. This role was further supported by the ability of a dominant-negative mutant of IkappaBalpha to block the IL-1 and TNF-alpha inhibition of **Sox9**-dependent **Col2a1** **enhancer** elements. Furthermore, forced expression of the NFkappaB subunits p65 or p50 also inhibited **Sox9**-dependent **Col2a1** **enhancer**. Because **Sox9** is essential for chondrogenesis, the marked down-regulation of the **Sox9** gene by IL-1 and TNF-alpha in chondrocytes is sufficient to account for the inhibition of the chondrocyte phenotype by these cytokines. The down-regulation of **Sox9** may have a crucial role in inhibiting expression of the cartilage phenotype in inflammatory joint diseases.

L11 ANSWER 6 OF 13 MEDLINE on STN
AN 1999251586 MEDLINE
TI **Sox9** is required for cartilage formation.
SO NATURE GENETICS, **(1999 May)** 22 (1) 85-9.
Journal code: 9216904. ISSN: 1061-4036.
AU Bi W; Deng J M; Zhang Z; Behringer R R; de Crombrugghe B
AB Chondrogenesis results in the formation of cartilages, initial skeletal elements that can serve as templates for endochondral bone formation. Cartilage formation begins with the condensation of mesenchyme cells followed by their differentiation into chondrocytes. Although much is known about the terminal differentiation products that are expressed by chondrocytes, little is known about the factors that specify the chondrocyte lineage. **SOX9** is a high-mobility-group (HMG) domain transcription factor that is expressed in chondrocytes and other tissues. In humans, **SOX9** haploinsufficiency results in campomelic dysplasia, a lethal skeletal malformation syndrome, and XY sex reversal. During embryogenesis, **Sox9** is expressed in all cartilage primordia and cartilages, coincident with the expression of the **collagen** alpha1(II) gene (**Col2a1**) . **Sox9** is also expressed in other tissues, including the central nervous and urogenital systems. **Sox9** binds to essential sequences in the **Col2a1** and **collagen** alpha2(XI) gene (Col11a2) chondrocyte-specific **enhancers** and can activate these **enhancers** in non-chondrocytic cells. Here, **Sox9** is identified as a regulator of the chondrocyte lineage. In mouse chimaeras, **Sox9**-/- cells are excluded from all cartilages but are present as a juxtaposed mesenchyme that does not express the chondrocyte-specific markers **Col2a1**, Col9a2, Col11a2 and Agc. This exclusion occurred cell autonomously at the condensing mesenchyme stage of chondrogenesis. Moreover, no cartilage developed in teratomas derived from **Sox9**-/- embryonic stem (ES) cells. Our results identify **Sox9** as the first transcription factor that is essential for chondrocyte differentiation and cartilage formation.

L11 ANSWER 7 OF 13 MEDLINE on STN
AN 1999077950 MEDLINE
TI Mechanism of regulatory target selection by the SOX high-mobility-group domain proteins as revealed by comparison of SOX1/2/3 and **SOX9**.
SO MOLECULAR AND CELLULAR BIOLOGY, **(1999 Jan)** 19 (1) 107-20.
Journal code: 8109087. ISSN: 0270-7306.
AU Kamachi Y; Cheah K S; Kondoh H
AB SOX proteins bind similar DNA motifs through their high-mobility-group (HMG) domains, but their action is highly specific with respect to target genes and cell type. We investigated the mechanism of target selection by comparing SOX1/2/3, which activate delta-crystallin minimal **enhancer** DC5, with **SOX9**, which activates **Col2a1** minimal **enhancer** COL2C2. These **enhancers** depend on both the SOX binding site and the binding site of a putative partner factor. The DC5 site was equally bound and bent by the HMG domains of SOX1/2 and **SOX9**. The activation domains of these SOX proteins mapped at the distal portions of the C-terminal domains were not cell specific and were independent of the partner factor. Chimeric proteins produced between SOX1 and **SOX9** showed that to activate the DC5 **enhancer**, the C-terminal domain must be that of SOX1, although the HMG domains were replaceable. The SOX2-VP16 fusion protein, in which the activation domain of SOX2 was replaced by that of VP16, activated the DC5 **enhancer** still in a partner factor-dependent manner. The results argue that the proximal portion of the C-terminal domain of SOX1/2 specifically interacts with the partner factor, and this interaction determines the specificity of the SOX1/2 action. Essentially the same results were obtained in the converse experiments in which COL2C2 activation by **SOX9** was analyzed, except that specificity of **SOX9**-partner factor interaction also involved the **SOX9** HMG domain. The highly selective SOX-partner factor interactions presumably stabilize the DNA binding of the SOX proteins and provide the mechanism for regulatory target selection.

L11 ANSWER 8 OF 13 MEDLINE on STN
AN 1998429495 MEDLINE
TI A new long form of Sox5 (L-Sox5), Sox6 and **Sox9** are coexpressed

in chondrogenesis and cooperatively activate the type II **collagen** gene.

SO EMBO JOURNAL, **(1998 Oct 1)** 17 (19) 5718-33.
Journal code: 8208664. ISSN: 0261-4189.

AU Lefebvre V; Li P; de Crombrugghe B

AB Transcripts for a new form of Sox5, called L-Sox5, and Sox6 are coexpressed with **Sox9** in all chondrogenic sites of mouse embryos. A coiled-coil domain located in the N-terminal part of L-Sox5, and absent in Sox5, showed >90% identity with a similar domain in Sox6 and mediated homodimerization and heterodimerization with Sox6. Dimerization of L-Sox5/Sox6 greatly increased efficiency of binding of the two Sox proteins to DNA containing adjacent HMG sites. L-Sox5, Sox6 and **Sox9** cooperatively activated expression of the chondrocyte differentiation marker **Col2a1** in 10T1/2 and MC615 cells. A 48 bp chondrocyte-specific **enhancer** in this gene, which contains several HMG-like sites that are necessary for **enhancer** activity, bound the three Sox proteins and was cooperatively activated by the three Sox proteins in non-chondrogenic cells. Our data suggest that L-Sox5/Sox6 and **Sox9**, which belong to two different classes of Sox transcription factors, cooperate with each other in expression of **Col2a1** and possibly other genes of the chondrocytic program.

L11 ANSWER 9 OF 13 MEDLINE on STN

AN 1998279015 MEDLINE

TI Chondrocyte-specific **enhancer** elements in the Col11a2 gene resemble the **Col2a1** tissue-specific **enhancer**.

SO JOURNAL OF BIOLOGICAL CHEMISTRY, **(1998 Jun 12)** 273 (24) 14998-5006.
Journal code: 2985121R. ISSN: 0021-9258.

AU Bridgewater L C; Lefebvre V; de Crombrugghe B

AB Type XI **collagen** and type II **collagen** are coexpressed in all cartilage, and both are essential for normal cartilage differentiation and skeletal morphogenesis. This laboratory has recently identified a 48-base pair (bp) **enhancer** element in the type II **collagen** gene **Col2a1** that contains several HMG-type protein-binding sites and that can direct chondrocyte-specific expression in transient transfection and in transgenic mice. The present study has identified two short chondrocyte-specific **enhancer** elements within a region in the 5' portion of the type XI **collagen** gene Col11a2 that has previously been shown to influence chondrocyte-specific expression in transgenic mice. These Col11a2 **enhancer** elements, like the **Col2a1 enhancer**, contain several sites with homology to the high mobility group (HMG) protein-binding consensus sequence. In electrophoretic mobility shift assays, the Col11a2 elements formed a DNA-protein complex that was dependent on the presence of the HMG-like sites. It had the same mobility as the complex formed with the **Col2a1** 48-bp **enhancer** and appeared to contain the same or similar proteins, including **SOX9**. The Col11a2 elements directed gene expression in transient transfections of chondrocytes but not fibroblasts, and their activity was abolished by mutation of the HMG-like sites. Ectopically expressed **SOX9** activated these **enhancers** in non-chondrocytic cells, as it also activates the **Col2a1 enhancer**. Finally, the Col11a2 **enhancer** elements both directed transgene expression to cartilage in developing mouse embryos. Overall, our results indicate that the two Col11a2 chondrocyte-specific **enhancer** elements share many similarities with the **Col2a1** 48-bp **enhancer**. These similarities suggest the existence of a genetic program designed to coordinately regulate the expression of these and perhaps other genes involved in the chondrocyte differentiation pathway.

L11 ANSWER 10 OF 13 MEDLINE on STN

AN 1998279014 MEDLINE

TI Three high mobility group-like sequences within a 48-base pair **enhancer** of the **Col2a1** gene are required for cartilage-specific expression in vivo.

SO JOURNAL OF BIOLOGICAL CHEMISTRY, **(1998 Jun 12)** 273 (24) 14989-97.
Journal code: 2985121R. ISSN: 0021-9258.

AU Zhou G; Lefebvre V; Zhang Z; Eberspaecher H; de Crombrugghe B

AB To understand the molecular mechanisms by which mesenchymal cells differentiate into chondrocytes, we have used the gene for an early and abundant marker of chondrocytes, the mouse pro-alpha1(II) **collagen** gene (**Col2a1**), to delineate a minimal sequence needed for chondrocyte-specific expression and to identify the DNA-binding proteins that mediate its activity. We show here that a 48-base pair (bp) **Col2a1** intron 1 sequence specifically targets the activity of a heterologous promoter to chondrocytes in transgenic mice. Mutagenesis studies of this 48-bp element identified three separate sites (sites 1-3) that were essential for its chondrocyte-specific **enhancer** activity in both transgenic mice and transient transfections. Mutations in sites 1 and 2 also severely inhibited the chondrocyte-specific **enhancer** activity of a 468-bp **Col2a1** intron 1 sequence in vivo. **SOX9**, an SRY-related high mobility group (HMG) domain transcription factor, was previously shown to bind site 3, to bend the 48-bp DNA at this site, and to strongly activate this 48-bp **enhancer** as well as larger **Col2a1 enhancer** elements. All three sites correspond to imperfect binding sites for HMG domain proteins and appear to be involved in the formation of a large chondrocyte-specific complex between the 48-bp element, **Sox9**, and other protein(s). Indeed, mutations in each of the three HMG-like sites of the 48-bp element, which abolished chondrocyte-specific expression of reporter genes in transgenic mice and in transiently transfected cells, inhibited formation of this complex. Overall our results suggest a model whereby both **Sox9** and these other proteins bind to several HMG-like sites in the **Col2a1** gene to cooperatively control its expression in cartilage.

L11 ANSWER 11 OF 13 MEDLINE on STN
AN 1998228078 MEDLINE
TI Toward understanding **SOX9** function in chondrocyte differentiation.
SO MATRIX BIOLOGY, **(1998 Mar)** 16 (9) 529-40. Ref: 50
Journal code: 9432592. ISSN: 0945-053X.
AU Lefebvre V; de Crombrugghe B
AB The transcription factors that trigger the determinative switch to chondrocyte differentiation in mesenchymal cells are still unknown. In humans, mutations in the gene for **SOX9**, a transcription factor with a DNA-binding domain similar to that of the mammalian testis-determining factor SRY, cause campomelic dysplasia, a severe dwarfism syndrome which affects all cartilage-derived structures. During mouse embryonic development, the **Sox9** gene becomes active in all prechondrocytic mesenchymal condensations, and at later stages its expression is maintained at high levels in fully differentiated chondrocytes. A chondrocyte-specific **enhancer** in the gene for **collagen** type II (**Col2a1**), a characteristic marker of chondrocytes, is a direct target for **SOX9**, and ectopic expression of **SOX9** in transgenic mouse embryos is sufficient to activate the endogenous **Col2a1** gene in some tissues. These data suggest that **SOX9** could have a major role in chondrogenesis. Studies are in progress to identify other target genes for **SOX9** in chondrocytes and also other transcription factors that are believed to cooperate with **SOX9** in the activation of chondrocyte-specific genes. Defining **SOX9** function and the mechanisms that regulate **SOX9** gene expression should contribute to a better understanding of chondrocyte differentiation.

L11 ANSWER 12 OF 13 MEDLINE on STN
AN 97407512 MEDLINE
TI Parallel expression of **Sox9** and **Col2a1** in cells undergoing chondrogenesis.
SO DEVELOPMENTAL DYNAMICS, **(1997 Aug)** 209 (4) 377-86.
Journal code: 9201927. ISSN: 1058-8388.
AU Zhao Q; Eberspaecher H; Lefebvre V; De Crombrugghe B
AB To assess the role of the transcription factor **Sox9** in cartilage formation we have compared the expression pattern of **Sox9** and **Col2a1** at various stages of mouse embryonic development. Expression of **Col2a1** colocalized with expression of **Sox9** in all chondroprogenitor cells. In the sclerotomal compartment of somites the onset of **Sox9** expression preceded that of **Col2a1**.

A perfect correlation was also seen between high levels of **Sox9** expression and high levels of **Col2a1** expression in chondrocytic cells. However, no **Sox9** expression was detected in hypertrophic chondrocytes; only low levels of **Col2a1** RNA were found in the upper hypertrophic zone. Coexpression of **Sox9** and **Col2a1** was also seen in the notochord. At E11.5 **Sox9** expression in the brain and spinal neural tube was more widespread than that of **Col2a1** although at E14.5 **Sox9** and **Col2a1** transcripts were colocalized in discrete areas of the brain. Distinct differences between **Sox9** and **Col2a1** expression were observed in the otic vesicle at E11.5. At E8.5, expression of **Sox9** but not of **Col2a1** was seen in the dorsal tips of the neural folds and after neural tube closure also in presumptive crest cells emigrating from the dorsal pole of the neural tube. No **Col2a1** expression was detected in gonadal ridges in which high levels of **Sox9** expression were detected. Together with our previous results showing that the chondrocyte-specific **enhancer** element of the **Col2a1** gene is a direct target for **Sox9**, these results suggest that **Sox9** plays a major role in expression of **Col2a1**. The correlation between high expression levels of **Sox9** and high expression levels of **Col2a1** in chondrocytes suggests the hypothesis that high levels of **Sox9** are needed for full expression of the chondrocyte phenotype; lower levels of **Sox9** such as in neuronal tissues which are also associated with lower expression levels of **Col2a1** would be compatible with other cell specifications.

L11 ANSWER 13 OF 13 MEDLINE on STN
AN 97220025 MEDLINE
TI **SOX9** is a potent activator of the chondrocyte-specific **enhancer** of the pro alpha1(II) **collagen** gene.
SO MOLECULAR AND CELLULAR BIOLOGY, **(1997 Apr)** 17 (4) 2336-46.
Journal code: 8109087. ISSN: 0270-7306.
AU Lefebvre V; Huang W; Harley V R; Goodfellow P N; de Crombrugghe B
AB The identification of mutations in the SRY-related **SOX9** gene in patients with campomelic dysplasia, a severe skeletal malformation syndrome, and the abundant expression of **Sox9** in mouse chondroprogenitor cells and fully differentiated chondrocytes during embryonic development have suggested the hypothesis that **SOX9** might play a role in chondrogenesis. Our previous experiments with the gene (**Col2a1**) for **collagen** II, an early and abundant marker of chondrocyte differentiation, identified a minimal DNA element in intron 1 which directs chondrocyte-specific expression in transgenic mice. This element is also a strong chondrocyte-specific **enhancer** in transient transfection experiments. We show here that **Col2a1** expression is closely correlated with high levels of **SOX9** RNA and protein in chondrocytes. Our experiments indicate that the minimal **Col2a1 enhancer** is a direct target for **Sox9**. Indeed, **SOX9** binds to a sequence of the minimal **Col2a1 enhancer** that is essential for activity in chondrocytes, and **SOX9** acts as a potent activator of this **enhancer** in cotransfection experiments in nonchondrocytic cells. Mutations in the **enhancer** that prevent binding of **SOX9** abolish **enhancer** activity in chondrocytes and suppress **enhancer** activation by **SOX9** in nonchondrocytic cells. Other SOX family members are ineffective. Expression of a truncated **SOX9** protein lacking the transactivation domain but retaining DNA-binding activity interferes with **enhancer** activation by full-length **SOX9** in fibroblasts and inhibits **enhancer** activity in chondrocytes. Our results strongly suggest a model whereby **SOX9** is involved in the control of the cell-specific activation of **COL2A1** in chondrocytes, an essential component of the differentiation program of these cells. We speculate that in campomelic dysplasia a decrease in **SOX9** activity would inhibit production of **collagen** II, and eventually other cartilage matrix proteins, leading to major skeletal anomalies.

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L9 ANSWER 5 OF 21 MEDLINE on STN
AN 1998279014 MEDLINE
TI Three high mobility group-like sequences within a 48-base pair **enhancer** of the **Col2a1** gene are required for cartilage-specific expression in vivo.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Jun 12) 273 (24) 14989-97. Journal code: 2985121R. ISSN: 0021-9258.
AU Zhou G; Lefebvre V; Zhang Z; Eberspaecher H; de Crombrugghe B
AB To understand the molecular mechanisms by which mesenchymal cells differentiate into chondrocytes, we have used the gene for an early and abundant marker of chondrocytes, the mouse pro-alpha1(II) **collagen** gene (**Col2a1**), to delineate a minimal sequence needed for chondrocyte-specific expression and to identify the DNA-binding proteins that mediate its activity. We show here that a 48-base pair (bp) **Col2a1** intron 1 sequence specifically targets the activity of a heterologous promoter to chondrocytes in transgenic mice. Mutagenesis studies of this 48-bp element identified three separate sites (sites 1-3) that were essential for its chondrocyte-specific **enhancer** activity in both transgenic mice and transient transfections. Mutations in sites 1 and 2 also severely inhibited the chondrocyte-specific **enhancer** activity of a 468-bp **Col2a1** intron 1 sequence in vivo. **SOX9**, an SRY-related high mobility group (HMG) domain transcription factor, was previously shown to bind site 3, to bend the 48-bp DNA at this site, and to strongly activate this 48-bp **enhancer** as well as larger **Col2a1 enhancer** elements. All three sites correspond to imperfect binding sites for HMG domain proteins and appear to be involved in the formation of a large chondrocyte-specific complex between the 48-bp element, **Sox9**, and other protein(s). Indeed, mutations in each of the three HMG-like sites of the 48-bp element, which abolished chondrocyte-specific expression of reporter genes in transgenic mice and in transiently transfected cells, inhibited formation of this complex. Overall our results suggest a model whereby both **Sox9** and these other proteins bind to several HMG-like sites in the **Col2a1** gene to cooperatively control its expression in cartilage.

L14 ANSWER 6 OF 19 MEDLINE on STN
AN 1999251586 MEDLINE
TI Sox9 is required for cartilage formation.
SO NATURE GENETICS, (1999 May) 22 (1) 85-9.
Journal code: 9216904. ISSN: 1061-4036.
AU Bi W; Deng J M; Zhang Z; Behringer R R; de Crombrugghe B
AB Chondrogenesis results in the formation of cartilages, initial skeletal elements that can serve as templates for endochondral bone formation. Cartilage formation begins with the condensation of mesenchyme cells followed by their differentiation into chondrocytes. Although much is known about the terminal differentiation products that are expressed by chondrocytes, little is known about the factors that specify the chondrocyte lineage. **SOX9** is a high-mobility-group (HMG) domain transcription factor that is expressed in chondrocytes and other tissues. In humans, **SOX9** haploinsufficiency results in campomelic dysplasia, a lethal skeletal malformation syndrome, and XY sex reversal. During embryogenesis, **Sox9** is expressed in all cartilage primordia and cartilages, coincident with the expression of the **collagen** alpha1(II) gene (**Col2a1**) . **Sox9** is also expressed in other tissues, including the central nervous and urogenital systems. **Sox9** binds to essential sequences in the **Col2a1** and **collagen** alpha2(XI) gene (Col11a2) chondrocyte-specific **enhancers** and can activate these **enhancers** in non-chondrocytic cells. Here, **Sox9** is identified as a regulator of the chondrocyte lineage. In mouse chimaeras, **Sox9**-/- cells are excluded from all cartilages but are present as a juxtaposed mesenchyme that does not express the chondrocyte-specific markers **Col2a1**, Col9a2, Col11a2 and Agc. This exclusion occurred cell autonomously at the condensing mesenchyme stage of chondrogenesis. Moreover, no cartilage developed in teratomas derived from **Sox9**-/- embryonic stem (ES) cells. Our results identify **Sox9** as the first transcription factor that is essential for chondrocyte differentiation and cartilage formation.

L14 ANSWER 5 OF 19 MEDLINE on STN
AN 1998228078 MEDLINE
TI Toward understanding SOX9 function in chondrocyte differentiation.
SO MATRIX BIOLOGY, (1998 Mar) 16 (9) 529-40. Ref: 50
Journal code: 9432592. ISSN: 0945-053X.
AU Lefebvre V; de Crombrugghe B
AB The transcription factors that trigger the determinative switch to chondrocyte differentiation in mesenchymal cells are still unknown. In humans, mutations in the gene for **SOX9**, a transcription factor with a DNA-binding domain similar to that of the mammalian testis-determining factor SRY, cause campomelic dysplasia, a severe dwarfism syndrome which affects all cartilage-derived structures. During mouse embryonic development, the **Sox9** gene becomes active in all prechondrocytic mesenchymal condensations, and at later stages its expression is maintained at high levels in fully differentiated chondrocytes. A chondrocyte-specific **enhancer** in the gene for **collagen** type II (**Col2a1**), a characteristic marker of chondrocytes, is a direct target for **SOX9**, and ectopic expression of **SOX9** in transgenic mouse embryos is sufficient to activate the endogenous **Col2a1** gene in some tissues. These data suggest that **SOX9** could have a major role in chondrogenesis. Studies are in progress to identify other target genes for **SOX9** in chondrocytes and also other transcription factors that are believed to cooperate with **SOX9** in the activation of chondrocyte-specific genes. Defining **SOX9** function and the mechanisms that regulate **SOX9** gene expression should contribute to a better understanding of chondrocyte differentiation.

L Number	Hits	Search Text	DB	Time stamp
1	37	sox9	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/11/14 17:52
2	3506	collagen?	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/11/14 17:38
3	87	col2a$2	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/11/14 17:38
6	9	sox9 and col2a$2	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/11/14 17:41
7	26	sox9 and chondro$9	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/11/14 17:42
16	1	Underhill WITH tully WITH Michael	USPAT; US-PGPUB; DERWENT	2003/11/14 17:51
17	29	sox9 and enhanc$9	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/11/14 17:53
18	11	(sox9 and enhanc$9) and (col1$3 or col2$3)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/11/14 17:54
19	7	(US-6610509-$ or US-6143878-$ or US-6316597-$).did. or (US-20020061514-$ or US-20020055480-$ or US-20020132239-$).did. or (CA-2357549-$).did.	USPAT; US-PGPUB; DERWENT	2003/11/14 17:55